Sporosarcina antarctica sp. nov., a psychrophilic bacterium isolated from the Antarctic

Yong Yu,¹ Yu-Hua Xin,² Hong-Can Liu,² Bo Chen,¹ Jun Sheng,³
Zhen-Ming Chi,³ Pei-Jin Zhou² and De-Chao Zhang³

Correspondence

De-Chao Zhang

zhangdechao@yahoo.cn

¹SOA Key Laboratory for Polar Science, Polar Research Institute of China, Shanghai 200136, PR China

²China General Microbiological Culture Collection Center and State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

³UNESCO Chinese Center of Marine Biotechnology, Ocean University of China, Qingdao 266003, PR China

A Gram-positive, psychrophilic, rod-shaped bacterium, designated strain N-05^T, was isolated from soil samples collected off King George Island, west Antarctica (62° 13' 31" S 58° 57' 08" W). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain N-05^T was related to members of the genus *Sporosarcina* and had highest 16S rRNA gene sequence similarity with the type strain of *Sporosarcina macmurdoensis* (98.0 %). The temperature range for growth of strain N-05^T was 0–23 °C, with optimum growth occurring at 17–18 °C and approximately pH 6.0–8.0. Strain N-05^T had MK-7 as the major menaquinone and anteiso-C_{15:0} and C_{16:1}ω7c alcohol as major fatty acids. The genomic DNA G + C content was 39.2 mol%. On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, strain N-05^T is considered to represent a novel species of the genus *Sporosarcina*, for which the name *Sporosarcina antarctica* is proposed. The type strain is N-05^T (=CGMCC 1.6503^T=JCM 14646^T).

The genus *Sporosarcina*, which belongs to the family *Bacillaceae*, was proposed by Kluyver & van Niel (1936) to accommodate bacteria that have spherical or oval-shaped cells and contain MK-7 as the major menaquinone. At the time of writing, the genus *Sporosarcina* comprises nine recognized species, namely *Sporosarcina ureae* (Claus *et al.*, 1983; Claus & Fahmy, 1986), *S. globispora* (Larkin & Stokes, 1967; Yoon *et al.*, 2001), *S. psychrophila* (Nakamura, 1984; Yoon *et al.*, 2001), *S. pasteurii* (Chester, 1898; Yoon *et al.*, 2001), *S. aquimarina* (Yoon *et al.*, 2001), *S. macmurdoensis* (Reddy *et al.*, 2003), *S. koreensis* and *S. soli* (Kwon *et al.*, 2007), and *S. saromensis* (An *et al.*, 2007). Here we report on the characterization of a novel psychrophilic bacterium of the genus *Sporosarcina* isolated from soil samples collected off King George Island, west Antarctica (62° 13' 31" S 58° 57' 08" W).

In the laboratory, frozen soil collected off King George Island was suspended in an equal volume of liquid PYG medium (0.5 % bacto peptone, 0.02 % yeast extract, 0.5 %

glucose, 0.3 % beef extract, 0.05 % NaCl and 0.15 % MgSO₄·7H₂O, w/v, pH adjusted to 7.0; Zhang *et al.*, 2007) supplemented with cycloheximide, nystatin and nalidixic acid (all at 25 µg ml^{−1}) and shaken at 10 °C for 4 days at 150 r.p.m. The culture was further diluted (1 : 10) and spread onto PYG agar plates and incubated at 4 °C for 2 weeks. Strain N-05^T was obtained in pure culture after three successive transfers to fresh PYG agar and stored at −80 °C in 30 % (v/v) glycerol. *S. macmurdoensis* DSM 15428^T was obtained from the DSMZ and routinely grown on ABM agar (0.5 % peptone, 0.2 % yeast extract and 1.5 % agar, w/v, pH 7.2) at 18 °C and used as a reference strain.

DNA was extracted and purified as described by Sambrook *et al.* (1989). The gene encoding 16S rRNA was amplified by PCR with two universal primers (Zhang *et al.*, 2007). Sequencing reactions were carried out by using an ABI BigDye 3.1 sequencing kit (Applied Biosystems) and an automated DNA sequencer (model ABI3730; Applied Biosystems). The nearly complete 16S rRNA gene sequence (1446 bp) of strain N-05^T was submitted to GenBank and EMBL to search for similar sequences by using the BLAST algorithm. A phylogenetic tree was constructed by using Kimura's two-parameter and pairwise-deletion model analysis implemented in the program MEGA version 3.0 (Kumar *et al.*, 2004). Resultant tree topologies were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N-05^T is EF154512.

A table detailing the cellular fatty acid content of strain N-05^T in comparison with other recognized *Sporosarcina* species is available as supplementary material with the online version of this paper.

evaluated by bootstrap analysis based on 1000 replicates. Phylogenetic analysis (Fig. 1) showed that strain N-05^T was grouped with members of the genus *Sporosarcina*, and formed a distinct cluster with *S. macmurdoensis* DSM 15428^T (98.0 % 16S rRNA gene sequence similarity) supported by a high bootstrap value (100 %) in the neighbour-joining tree. Levels of 16S rRNA gene sequence similarity between strain N-05^T and other strains used in the phylogenetic analysis were less than 95.6 %. A similar topology was found in the tree generated with the maximum-parsimony algorithm (data not shown).

Cell morphology of strain N-05^T was examined with an Axioplan 2 microscope. Colony morphology was observed following incubation on PYG at 17 °C. Growth temperature was determined with a TN3F temperature-gradient incubator (Advantec). The pH range for growth was determined by using PYG broth at various pH values adjusted with HCl or NaOH (both at 1 mol l⁻¹). Anaerobic growth was investigated in PYG medium in an anaerobic test tube filled with nitrogen gas. General physiological tests were performed by using conventional methods (Dong & Cai, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). API strips (API 20 E, API 20 NE, API ZYM; bioMérieux) were used to determine the physiological and biochemical characteristics of strain N-05^T according to the manufacturer's instructions. The API strips were incubated for 4 days at 17 °C. All of the tests were performed in duplicate. Morphological, physiological and biochemical characteristics of strain N-05^T are given in the species description below or are shown in Table 1.

Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989) by using menaquinone 7 (MK-7) from *S. macmur-*

doensis DSM 15428^T as a reference. Cell-wall peptidoglycan was prepared and analysed in accordance with the methods of Komagata & Suzuki (1987) and Zhang *et al.* (2007). For analysis of fatty acid methyl esters, cell mass of strain N-05^T was harvested from PYG plates after incubation at 17 °C for 4 days. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI (Microbial Identification) system (Sasser, 1990). Strain N-05^T contained MK-7 as the major menaquinone. The peptidoglycan of the isolate contained a cell-wall type of L-Lys-D-Glu (variation A4α). The predominant cellular fatty acids of strain N-05^T were anteiso-C_{15:0} (39.75 % of the total), C_{16:1}ω7c alcohol (18.89 %), iso-C_{17:1} I and/or anteiso-C_{17:1} B (11.91 %), iso-C_{14:0} (7.55 %), iso-C_{16:0} (7.01 %), anteiso-C_{17:0} (4.23 %), C_{16:1}ω11c (3.67 %), iso-C_{15:0} (1.36 %), iso-C_{16:1} (1.30 %) and C_{16:0} (1.19 %). The fatty acid profile of strain N-05^T was similar to those of recognized *Sporosarcina* species (Yoon *et al.*, 2001), although strain N-05^T contained a greater proportion of unsaturated fatty acids (C_{16:1}ω7c alcohol, iso-C_{17:1} I and/or anteiso-C_{17:1} B), which contribute to the maintenance of membrane fluidity at low temperature. The fatty acid profiles of strain N-05^T and other recognized *Sporosarcina* species are detailed in Supplementary Table S1 available in IJSEM Online.

The DNA G+C content was determined by using the thermal denaturation method with *Escherichia coli* K-12 as reference, and DNA-DNA hybridization experiments were performed using the liquid renaturation method (De Ley *et al.*, 1970) as modified by Huß *et al.* (1983); both experiments were carried out by using a DU800 spectrophotometer (Beckman) with a thermal controller. The DNA G+C content of strain N-05^T was 39.2 mol%. The level of DNA-DNA relatedness between strain N-05^T and *S. macmurdoensis* DSM 15428^T was as low as 41.7 %, much

Fig. 1. Phylogenetic relationships between strain N-05^T, recognized members of the genus *Sporosarcina* and other related species based on 16S rRNA gene sequences. The tree was constructed by using the neighbour-joining method. Numbers at nodes represent levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. GenBank accession numbers are given in parentheses. Bar, 1 % sequence divergence.

Table 1. Differential phenotypic characteristics between strain N-05^T and recognized *Sporosarcina* species

Strains: 1, N-05^T (*S. antarctica* sp. nov.); 2, *S. macmurdoensis* CMS 21w^T (data from Reddy *et al.*, 2003); 3, *S. aquimarina* SW28^T (Yoon *et al.*, 2001); 4, *S. globispora* DSM 4^T (Nakamura, 1984; Rüger, 1983); 5, *S. psychrophila* IAM 12468^T (Nakamura, 1984); 6, *S. pasteurii* NCIMB 8841^T (Yoon *et al.*, 2001); 7, *S. ureae* DSM 2281^T (Claus & Fahmy, 1986); 8, *S. koreensis* F73^T (Kwon *et al.*, 2007); 9, *S. soli* I80^T (Kwon *et al.*, 2007); 10, *S. saromensis* HG645^T (An *et al.*, 2007). +, Positive; –, negative; w, weak; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Colony colour*	LY	WH	LO	WH	WH	WH	WH	LO	LO	BE
Cell shape†	R	R	R	R	R	S	S	R	R	R
Motility	–	–	+	+	+	+	+	+	–	+
Growth at:										
5 °C	+	+	+	+	+	NA	NA	–	–	+
30 °C	–	–	+	+	w	+	+	+	+	+
Anaerobic growth	+	+	+	+	–	+	–	–	–	–
Presence of:										
Oxidase	+	–	+	+	+	NA	+	+	+	+
Urease	–	–	+	+	+	+	+	+	+	+
NaCl tolerance (%)	9	3	13	5	2	10	3	7	5	9
Hydrolysis of:										
Casein	–	NA	–	–	w	w	–	–	–	–
Gelatin	–	+	+	+	+	+	–	+	–	+
Starch	–	+	–	–	w	–	–	–	–	+
Nitrate reduction	–	–	+	+	–	+	+	–	+	–
Acid production from:										
D-Glucose	–	–	–	+	+	NA	NA	–	–	–
D-Xylose	–	–	–	–	+	NA	NA	–	–	–
D-Fructose	–	–	+	–	+	NA	NA	NA	NA	–
D-Galactose	–	–	–	–	+	NA	NA	NA	NA	–
D-Mannitol	–	–	–	–	+	NA	NA	–	–	–
DNA G + C content (mol%)	39.2	44	40	40	44.1	39	40–42	46.5	44.5	46

*BE, Beige; LO, light orange; LY, light yellow; WH, white.

†R, Rods; S, spherical.

lower than the threshold (70 %) recommended for species demarcation (Wayne *et al.*, 1987), thus verifying that strain N-05^T represents a species distinct from *S. macmurdoensis*. 16S rRNA gene sequence similarities of less than 97.0 % were found between strain N-05^T and the type strains of other recognized species of the genus *Sporosarcina*. It has been suggested that in bacterial strains showing less than 97 % 16S rRNA gene sequence similarity, levels of DNA–DNA hybridization are less than 70 % (Stackebrandt & Goebel, 1994). Therefore, strain N-05^T is genotypically distinct from all recognized species of the genus *Sporosarcina*.

Based on the phenotypic, phylogenetic and genomic evidence presented, strain N-05^T is considered to represent a novel species of the genus *Sporosarcina*, for which the name *Sporosarcina antarctica* sp. nov. is proposed.

Description of *Sporosarcina antarctica* sp. nov.

Sporosarcina antarctica (ant.arc'ti.ca. L. fem. adj. *antarctica* southern, of the Antarctic, the geographical origin of the type strain).

Cells are Gram-positive, psychrophilic, non-motile rods, 0.3–0.6 µm by 1.1–2.6 µm in size. Colonies are light yellow, smooth, circular and convex with entire margins. Produces catalase and cytochrome oxidase. Growth occurs under anaerobic conditions on PYG medium. Growth occurs at 0–23 °C and pH 5.0–10.0, with optimum growth at 17–18 °C and approximately pH 6.0–8.0. Growth occurs in the presence of 0–9 % (w/v) NaCl. Nitrate is not reduced and hydrogen sulfide is not produced. Negative for indole production and the Voges–Proskauer reaction. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, leucine arylamidase, valine arylamidase, cystine arylamidase, urease, gelatinase, caseinase, trypsin, amylase, lecithinase, acid phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase and α-chymotrypsin. Positive for naphthol-AS-BI-phosphohydrolase and alkaline phosphatase. D-Glucose and α-ketobutyric acid can be utilized as sole carbon sources, but not sucrose, lactose, galactose, trehalose, cellobiose, L-rhamnose, melibiose, raffinose, D-sorbitol,

inositol, maltose, glucose, glycerol, fructose, melezitose, L-arabinose, D-mannose, mannitol, N-acetylglucosamine, malate, succinate, capric acid, adipic acid or phenylacetic acid. Acid is not produced from the following carbohydrates: D-glucose, fructose, sucrose, lactose, galactose, trehalose, cellobiose, L-rhamnose, melibiose, raffinose, D-sorbitol, inositol, maltose, glycerol, melezitose, L-arabinose, D-mannose or mannitol. MK-7 is the major menaquinone. The cell wall contains peptidoglycans of the L-Lys-D-Glu type (variation A4 α). Predominant cellular fatty acids are anteiso-C_{15:0} (39.75 % of the total), C_{16:1} ω 7c alcohol (18.89 %), iso-C_{17:1} I and/or anteiso-C_{17:1} B (11.91 %), iso-C_{14:0} (7.55 %), iso-C_{16:0} (7.01 %), anteiso-C_{17:0} (4.23 %) and C_{16:1} ω 11c (3.67 %). The DNA G + C content of the type strain is 39.2 mol%.

The type strain, N-05^T (=CGMCC 1.6503^T=JCM 14646^T), was isolated from soil samples collected off King George Island, west Antarctica (62° 13' 31" S 58° 57' 08" W).

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References

- An, S.-Y., Haga, T., Kasai, H., Goto, K. & Yokota, A. (2007). *Sporosarcina saromensis* sp. nov., an aerobic endospore-forming bacterium. *Int J Syst Evol Microbiol* **57**, 1868–1871.
- Chester, F. D. (1898). Report of the mycologist: bacteriological work. *Del Agric Exp Stn Annu Rep* **10**, 47–137.
- Claus, D. & Fahmy, F. (1986). Genus *Sporosarcina* Kluyver and van Niel 1936, 401^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1202–1206. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Claus, D., Fahmy, F., Rolf, H. J. & Tosunoglu, N. (1983). *Sporosarcina halophila* sp. nov., an obligate, slightly halophilic bacterium from salt marsh soils. *Syst Appl Microbiol* **4**, 496–506.
- Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Dong, X.-Z. & Cai, M.-Y. (editors) (2001). Determination of biochemical properties. In *Manual for the Systematic Identification of General Bacteria*, pp. 370–398. Beijing: Science Press (in Chinese).
- HuB, V. A. R., Festl, H. & Schleifer, K.-H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kluyver, A. J. & van Niel, C. B. (1936). Prospects for a natural classification of bacteria. *Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt II* **94**, 369–403.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kwon, S.-W., Kim, B.-Y., Song, J., Weon, H.-Y., Schumann, P., Tindall, B. J., Stackebrandt, E. & Fritze, D. (2007). *Sporosarcina koreensis* sp. nov. and *Sporosarcina soli* sp. nov., isolated from soil in Korea. *Int J Syst Evol Microbiol* **57**, 1694–1698.
- Larkin, J. M. & Stokes, J. L. (1967). Taxonomy of psychrophilic strains of *Bacillus*. *J Bacteriol* **94**, 889–895.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Nakamura, L. K. (1984). *Bacillus psychrophilus* sp. nov., nom. rev. *Int J Syst Bacteriol* **34**, 121–123.
- Reddy, G. S. N., Matsumoto, G. I. & Shivaji, S. (2003). *Sporosarcina macmurdoensis* sp. nov., from a cyanobacterial mat sample from a pond in the McMurdo Dry Valleys, Antarctica. *Int J Syst Evol Microbiol* **53**, 1363–1367.
- Rüger, H.-J. (1983). Differentiation of *Bacillus globisporus*, *Bacillus marinus* comb. nov., *Bacillus aminovorans*, and *Bacillus insolitus*. *Int J Syst Bacteriol* **33**, 157–161.
- Sambrook, J., Frisch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology* [English translation of *Microbiology* (Beijing)] **16**, 176–178.
- Yoon, J.-H., Lee, K.-C., Weiss, N., Kho, Y. H., Kang, K. H. & Park, Y.-H. (2001). *Sporosarcina aquimarina* sp. nov., a bacterium isolated from seawater in Korea, and transfer of *Bacillus globisporus* (Larkin and Stokes 1967), *Bacillus psychrophilus* (Nakamura 1984) and *Bacillus pasteurii* (Chester 1898) to the genus *Sporosarcina* as *Sporosarcina globispora* comb. nov., *Sporosarcina psychrophila* comb. nov. and *Sporosarcina pasteurii* comb. nov., and emended description of the genus *Sporosarcina*. *Int J Syst Evol Microbiol* **51**, 1079–1086.
- Zhang, D.-C., Wang, H.-X., Cui, H.-L., Yang, Y., Liu, H.-C., Dong, X.-Z. & Zhou, P.-J. (2007). *Cryobacterium psychrotolerans* sp. nov., a novel psychrotolerant bacterium isolated from the China No. 1 glacier. *Int J Syst Evol Microbiol* **57**, 866–869.